

REMARKS

Any fees that may be due in connection with filing this paper or with this application during its entire pendency may be charged to Deposit Account No. 06-1050. If a Petition for extension of time is required, this paper is to be considered such Petition, and any fee charged to Deposit Account No. 06-1050. A change of correspondence address form for the undersigned accompanies this response.

Paragraph 1 in the specification is amended for clarity to distinguish between U.S. Applications to which the instant application claims priority and those to which the instant application is related by subject matter and to which no priority claim is made.

Claims 17-22, 31, 33 and 35-41 are pending in this application. Claims 17, 18 and 22 are amended. Claim 34, which is withdrawn from consideration, is cancelled herein without prejudice or disclaimer. Applicant reserves the right to file divisional or continuation applications to any canceled claims or unclaimed subject matter disclosed in the specification. Claim 17 is amended herein to more particularly point out the subject matter by reciting that the nucleic acid molecule is a large nucleic acid molecule. Basis for the amendment of claim 17 can be found throughout the specification, which provides methods for labeling and introducing large nucleic acid molecules into a cell, including methods of detecting delivery of labeled large nucleic acids. Particular basis can be found, for example, on page 18, which recites: "A variety of methods for delivering nucleic acids, particularly large nucleic acids, such as artificial chromosomes, including ACes (formerly designated SATACS) are provided" (lines 2-4).

Particular basis for "large nucleic acid molecules" also can be found, for example, at page page 10, lines 5-22, which defines "large nucleic acids molecules as follows:

As used herein, the term "large nucleic acid molecules" or "large nucleic acids" refers to a nucleic acid molecule of at least about 0.5 megabase pairs (Mbase) in size, greater than 0.5 Mbase, including nucleic acid molecules at least about 0.6, 0.7, 0.8, 0.9, 1, 5, 10, 30, 50 and 100, 200, 300, 500 Mbase in size. Large nucleic acid molecules typically may be on the order of about 10 to about 450 or more Mbase, and may be of various sizes, such as, for example, from about 250 to about 400 Mbase, about 150 to about 200 Mbase, about 90 to about 120 Mbase, about 60 to about 100 Mbase and about 15 to 50 Mbase.

Examples of large nucleic acid molecules include, but are not limited to, natural chromosomes and fragments thereof, especially mammalian chromosomes and fragments thereof which retain a centromere and telomeres, artificial chromosome expression systems (ACes; also called satellite DNA-based artificial chromosomes (SATACs); see U.S. Patent Nos. 6,025,155 and 6,077,697), mammalian artificial chromosomes (MACs), plant artificial chromosomes, insect artificial chromosomes, avian artificial chromosomes and minichromosomes (see, e.g., 5,712,134, 5,891,691 and 5,288,625).

Labeling of large nucleic acid molecules, introduction of labeled large nucleic acid molecules into cells and detection of labeled large nucleic acid molecules are also exemplified in Examples 4 and 7.

The Examiner indicated in the instant office action that claims 18-21 would be allowable subject matter if rewritten as an independent claim incorporating all the limitations of an allowable base claim and any intervening claims. Accordingly, claim 18 is rewritten in independent form. Thus, claim 18 as amended is unchanged in scope from claim 18 as originally filed. Claim 22 is amended to change the order of members in the Markush group for clarity.

Claims 35-41 are added. These claims find basis throughout the specification and in the claims as originally filed. For example, basis for claims 35-41, can be found in the section of the specification entitled "D. Assessing the delivery of nucleic acids into cells" at pages 37-42, and for example in Examples 4-6 at pages 55-61 and Example 9 at pages 65-70.

Priority and objection to the Specification

The Office Action alleges that Applicant has improperly claimed priority to U.S. Application No. 09/815,979 by reciting that the instant application is "related to" U.S. Application No. 09/815,979 in the first paragraph of the application. The Office Action objects to this sentence in the specification and requests that this reference be removed or corrected.

Applicant respectfully submits that no claim of priority to U.S. Application No. 09/815,979 is made by the instant application. Applicant has used the language of "related to" to indicate a subject matter relationship between the instant application and U.S. Application No. 09/815,979. Although the MPEP specifies particular language to be used within the first

paragraph of the application for claiming priority, it does not preclude Applicant from statements directed towards other matters when no such priority claim is made.

For clarity, the specification is amended to remove the sentence referring to U.S. Application No. 09/815,979 and place it in a separate second paragraph. This amendment also amends the phrase "related to" to "related to the subject matter of" for further clarity. Accordingly, Applicant respectfully requests that this objection be withdrawn.

Information Disclosure Statement and Duty of Disclosure

Applicant acknowledges the signed and initial copied of the PTO-1149 forms sent by the Examiner and also thanks the Examiner for pointing out the references containing browser executable code. Replacement pages of the PTO-1149 forms at issue were faxed to the Examiner March 3, 2004, containing the references with the browser executable code removed.

The Examiner's attention is drawn to the co-pending U.S. applications serial nos. 09/815,979 and 09/815,981, which are of record in this application. Enclosed herewith are copies of the Office Actions that issued in connection with these applications and copies of the pending claims in the applications. The Examiner's attention is also drawn to the Supplemental Information Disclosure filed on the same day herewith under separate cover.

REJECTION OF CLAIM 22 UNDER 35 U.S.C. § 112

Claim 22 is rejected under 35 U.S.C. § 112, second paragraph as being indefinite. Specifically, it is alleged that the phrase "natural chromosome, artificial chromosome or naked DNA that is greater than about 0.6 megabases in size" is indefinite because it is unclear if the limitation "greater than about 0.6 megabases in size" applies to each member of the Markush group or only naked DNA. Reconsideration is respectfully requested.

Claim 22 as amended herein changes the order of the members of the Markush group for clarity. The amended claim recites "a naked DNA that is greater than about 0.6 megabases in size, a natural chromosome, an artificial chromosome or a fragment of a chromosome." The amendment agrees with the Examiner's interpretation of the claim; only the naked DNA is

restricted to a size greater than 0.6 megabases. Thus, the rejection is rendered moot and Applicant respectfully requests that this rejection be withdrawn.

REJECTION OF CLAIMS 17, 31 and 33 UNDER 35 U.S.C. § 102(b)

A. Claims 17 and 31 are rejected under 35 U.S.C. §102(b) as being anticipated by Felgner et al. (WO 99/13719 (1999)).

The Office Action alleges that Felgner *et al.* discloses a method of transfecting fluorescently-labeled plasmid into a cell and detecting the label by fluorescence microscopy. It also is alleged Felgner *et al.* describes the use of tumor cell lines for delivery and detection of labeled DNA. Reconsideration is respectfully requested.

RELEVANT LAW

Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration. In re Spada, 15 USPQ2d 1655 (Fed. Cir, 1990), In re Bond, 15 USPQ 1566 (Fed. Cir. 1990), Soundsciber Corp. v. U.S., 360 F.2d 954, 148 USPQ 298, 301, adopted 149 USPQ 640 (Ct. Cl.) 1966. See, also, Richardson v. Suzuki Motor Co., 868 F.2d 1226, 1236, 9 USPQ2d 1913,1920 (Fed. Cir.), cert. denied, 110 S.Ct. 154 (1989). "[A]ll limitations in the claims must be found in the reference, since the claims measure the invention." In re Lang, 644 F.2d 856, 862, 209 USPQ 288, 293 (CCPA 1981). Moreover it is incumbent on Examiner to identify wherein each and every facet of the claimed invention is disclosed in the reference. Lindemann Maschinen-fabrik GmbH v. American Hoist and Derrick Co., 730 F.2d 1452, 221 USPQ 481 (Fed. Cir. 1984). Further, the reference must describe the invention as claimed sufficiently to have placed a person of ordinary skill in the art in possession of the invention. An inherent property has to flow naturally from what is taught in a reference In re Oelrich, 666 F.2d 578, 581, 212 USPQ 323, 326 (CCPA 1981).

"Rejections under 35 U.S.C. §102 are proper only when the claimed subject matter is identically disclosed or described in the "prior art" . . .the [r]eference must clearly and unequivocally disclose the claimed compound or direct those skilled in the art to the compound without any need for picking, choosing, and combining various disclosures not directly related to

each other by the teachings in the cited references. Such picking and choosing may be entirely proper when making a rejection of a §103, obviousness rejection, where the applicant must be afforded an opportunity to rebut with objective evidence any inference of obviousness which may arise from the similarity of the subject matter which he claims to the prior art, but it has no place in the making of a §102, anticipation rejection." (Emphasis in original). In re Arkey, Eardly, and Long, 455 F.2d 586, 172 USPQ 524 (CCPA, 1972).

THE CLAIMS

Claim 17 is directed to a method of monitoring the delivery of a large nucleic acid molecule into a cell. The steps of the method include: a) labeling the large nucleic acid molecule; b) delivering labeled large nucleic acid molecule into a cell; c) detecting labeled nucleic acid molecule in the cells by flow cytometry, fluorimetry, cell imaging or fluorescence spectroscopy, as an indication of delivery of nucleic acid molecule into the cells. Dependent claim 31 further specifies that the cell is selected from the group consisting of a primary cell, an immortalized cell, an embryonic cell, a stem cell, a transformed cells and a tumor cell.

ANALYSIS

Felgner *et al.* discloses methods for labeling plasmid DNA with peptide nucleic acid (PNA) molecules. Felgner *et al.* further describes introducing PNA-plasmid DNA hybrids into cells. The plasmids of Felgner *et al.* are on the order of 10 kB. In the method of Felgner *et al.*, PNA is hybridized in a sequence-specific manner to the plasmid. In some embodiments of Felgner *et al.*, the PNA is conjugated to a label, exemplified by a rhodamine-PNA conjugate.

Felgner *et al.* does not disclose methods for monitoring the delivery of large nucleic acid molecules. The plasmid DNA molecules of Felgner *et al.* are not large nucleic acids. As described in the instant application, large nucleic acids are "at least about 0.5 megabases" (*i.e.*, about 500 kB; page 10, lines 5-14). Thus, Felgner *et al.* does not disclose any methods for labeling large nucleic acid molecules. Felgner *et al.* also does not disclose any methods for delivering a labeled large nucleic acid molecule into a cell, nor detecting labeled large nucleic acid molecules in a cell. Felgner *et al.* also does not disclose delivery or detection of labeled

large nucleic acids in any cell types including a primary cell, an immortalized cell, an embryonic cell, a stem cell, a transformed cell or a tumor cell (claim 31).

Since anticipation requires that a reference disclose all elements as claimed, Felgner *et al.*, which does not disclose labeling of large nucleic acid molecules, delivery of a labeled large nucleic acid into a cell, nor the detection of a labeled large nucleic acid molecule in a cell, does not anticipate claims 17 and 31.

B. Claims 17, 31 and 33 are rejected under 35 U.S.C. §102(b) as being anticipated by Zelphati *et al.* (1999) Hum Gene Ther. 10(1):15-24.

The Office Action alleges that Zelphati *et al.* discloses transfecting fluorescently-labeled DNA into cells and quantifying the number of cells containing fluorescent label using fluorescence microscopy. It is also alleged that this reference uses transformed cell lines for delivery and detection of labeled DNA. This rejection is respectfully traversed.

RELEVANT LAW

See above.

CLAIMS

Claims 17 and 31 are described above. Claim 33 further recites the step of determining the number of cells containing the label.

ANALYSIS

Zelphati *et al.* discloses a method of hybridizing rhodamine-labeled peptide nucleic acid molecules (PNA) to plasmid DNA. Zelphati *et al.* also discloses the delivery of PNA-labeled plasmid DNA to cells and the visualization of the labeled plasmid within cells. The plasmids used in Zelphati *et al.* range in size from about 5 kB to about 8 kB (page 2, column 2, first paragraph).

Zelphati *et al.* does not disclose methods for monitoring the delivery of large nucleic acid molecules. The plasmid DNA molecules labeled and delivered by Zelphati *et al.* are not large nucleic acid molecules. Thus, Zelphati *et al.* does not disclose labeling of large nucleic acid molecules. Zelphati *et al.* also does not disclose delivery of labeled large nucleic acids into cells or detection of labeled large nucleic acids. Further, Zelphati *et al.* does not disclose delivery or

detection of labeled large nucleic acids into cells including a primary cell, an immortalized cell, an embryonic cell, a stem cell, a transformed cell or a tumor cell (claim 31). Zelphati *et al.* also does not disclose the determination of the number of cells containing label from labeled large nucleic acid molecules (claim 33). Therefore, since anticipation requires that a reference disclose all elements as claimed, Zelphati *et al.*, which does not disclose labeling of a large nucleic acid molecule, nor the delivery of a labeled large nucleic acid into cells, nor the detection of labeled large nucleic acid in cells, does not anticipate any of the rejected claims 17, 31 and 33.

C. Claims 17, 31 and 33 are rejected under 35 U.S.C. §102(b) as being anticipated by Neves *et al.* ((1999/2000) Bioconjugate Chem. 11: 51-55).

The Office Action alleges that Neves *et al.* teaches covalent labeling of DNA with a fluorescent label and introduction of the labeled DNA into NIH 3T3 cells and CV-1 transformed cells. The Office Action further alleges that following transfection, Neves *et al.* determines the number of cells containing the fluorescent label by fluorescence microscopy.

RELEVANT LAW

See above.

CLAIMS

Claims 17, 31 and 33 are described above.

ANALYSIS

Neves *et al.* discloses the labeling of plasmid DNA with p-azido-tetrafluorobenzylamido-lissamine and with rhodamine nucleotides. Neves *et al.* discloses the introduction of these labeled plasmids and the detection of labeled cells by fluorescence microscopy. The plasmids labeled by the method of Neves *et al.* are about 7 kb (see Figure 1, page 53).

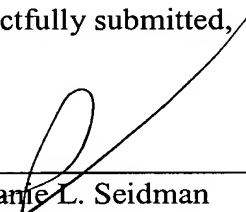
Neves *et al.* does not disclose a method for monitoring delivery of a large nucleic acid molecule. The plasmids disclosed in Neves *et al.* are not large nucleic acid molecules. Thus, Neves *et al.* does not disclose labeling large nucleic acid molecules. Neves *et al.* also does not disclose delivering labeled large nucleic acids into cells, nor detecting labeled large nucleic acid molecules as an indication of nucleic acid delivery into cells. Further, Neves *et al.* does not deliver or detect labeled large nucleic acids in any cells types including a primary cell, an

immortalized cell, an embryonic cell, a stem cell a transformed cell or a tumor cell (claim 31). Neves *et al.* also does not disclose the determination of the number of cells containing label from labeled large nucleic acid molecules (claim 33). Therefore, since anticipation requires that a reference disclose all elements as claimed, Neves *et al.*, which does not disclose labeling of a large nucleic acid molecule, nor delivery of a labeled large nucleic acid into a cell, nor detection of labeled large nucleic acid, does not anticipate claims 17, 31 and 33.

* * *

Entry of this amendment and examination of the application are respectfully requested.

Respectfully submitted,



Stephanie L. Seidman
Reg. No. 33,779

Fish & Richardson P.C.
12390 El Camino Real
San Diego, California 92130
Telephone: (858) 678-4777
Facsimile: (858) 678-5099



U.S. Application Serial No. 09/815,979 Pending Claims:

1. (currently amended) A method for introducing a large nucleic acid molecule into a cell, comprising:
 - (a) exposing a large nucleic acid molecule to a delivery agent;
 - (b) exposing the cell to a delivery agent; and
 - (c) contacting the cell with the nucleic acid molecule, whereby the nucleic acid molecule is delivered into the cell, wherein steps (a)-(c) are performed sequentially in any order, provided that if the delivery agent is energy it is not applied to the nucleic acid molecule and it is not applied to the cell after contacting the cell with the nucleic acid molecule.
2. (original) The method of claim 1, wherein:
 - the nucleic acid molecule is exposed to an agent that increases contact between the nucleic acid molecule and the cell; and
 - the cell is exposed to an agent that enhances permeability of the cell.
3. (original) The method of claim 1, wherein the nucleic acid molecule is greater than about 0.6 megabase.
4. (original) The method of claim 1, wherein the nucleic acid molecule is greater than about 1 megabase.
5. (original) The method of claim 1, wherein the nucleic acid molecule is greater than about 5 megabases.
6. (previously presented) The method of claim 1, wherein the nucleic acid molecule is a natural chromosome, an artificial chromosome, a fragment of a chromosome that is greater than about 0.6 megabase or naked DNA that is greater than about 0.6 megabase.
7. (original) The method of claim 1, wherein the nucleic acid molecule is an artificial chromosome.
8. (previously presented) The method of claim 1, wherein the nucleic acid molecule is an artificial chromosome expression system (ACes).
9. (currently amended) The method of claim 1, wherein the nucleic acid molecule is exposed to the delivery agent *in vitro*, or *ex vivo* or *in vivo*.
10. (original) The method of claim 1, wherein the contacting of the nucleic acid molecule that has been exposed to the delivery agent with the cell is effected in *vitro*, *ex vivo* or *in vivo*.
11. (original) The method of claim 1, wherein
 - exposure of the nucleic acid to a delivery agent is effected by mixing the nucleic acid with a delivery agent; and
 - the exposure of the cell to an agent that enhances permeability comprises applying ultrasound or electrical energy to the cell.
12. (original) The method of claim 1, wherein a delivery agent comprises a cationic compound.
13. (original) The method of claim 12, wherein the cationic compound is selected from the group consisting of a cationic lipid, a cationic polymer, a mixture of cationic lipids, a mixture of cationic polymers, a mixture of a cationic lipid and a cationic polymer, a mixture of a cationic lipid and a neutral lipid, polycationic lipids,

non-liposomal forming lipids, activated dendrimers, and a pyridinium chloride surfactant.

14. (previously presented) The method of claim 12, wherein the delivery agent is a composition that comprises one or more cationic compounds, wherein the compound is selected from the group consisting of N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), dioleoylphosphatidylethanolamine (DOPE), 2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminiumtrifluoroacetate (DOSPA), $C_{52}H_{106}N_6O_4 \cdot 4CF_3CO_2H$, $C_{88}H_{178}N_8O_4S_2 \cdot 4CF_3CO_2H$, $C_{40}H_{84}NO_3P \cdot CF_3CO_2H$, $C_{50}H_{103}N_7O_3 \cdot 4CF_3CO_2H$, $C_{55}H_{116}N_8O_2 \cdot 6CF_3CO_2H$, $C_{49}H_{102}N_6O_3 \cdot 4CF_3CO_2H$, $C_{44}H_{89}N_5O_3 \cdot 2CF_3CO_2H$, $C_{100}H_{206}N_{12}O_4S_2 \cdot 8CF_3CO_2H$, $C_{41}H_{78}NO_8P$, $C_{162}H_{330}N_{22}O_9 \cdot 13CF_3CO_2H$, $C_{43}H_{88}N_4O_2 \cdot 2CF_3CO_2H$, $C_{43}H_{88}N_4O_3 \cdot 2CF_3CO_2H$, and (1-methyl-4-(1-octadec-9-enyl-nonadec-10-enylenyl) pyridinium chloride.

15. (original) The method of claim 1, wherein a delivery agent is energy.

16. (original) The method of claim 15, wherein the cell is treated with energy.

17. (original) The method of claim 15, wherein the energy is ultrasound energy.

18. (original) The method of claim 17, wherein the ultrasound energy is applied to the cell for about 30 seconds to about 5 minutes.

19. (original) The method of claim 17, wherein the ultrasound energy is applied as one continuous pulse.

20. (original) The method of claim 17, wherein the ultrasound energy is applied as two or more intermittent pulses.

21. (original) The method of claim 20, wherein the intermittent pulses of the ultrasound energy are applied for substantially the same length of time, at substantially the same energy level.

22. (original) The method of claim 20, wherein the intermittent pulses vary in energy level, the length of time applied, or energy level and the length of time applied.

23. (original) The method of claim 11, wherein prior to applying the ultrasound energy to the cell, the cell is contacted with a cavitation compound.

24. (original) The method of claim 17, wherein prior to applying the ultrasound energy to the cell, the cell is contacted with a cavitation compound

25. (original) The method of claim 11, wherein the agent that enhances permeability comprises applying electrical energy.

26. (original) The method of claim 1 that comprises:

(a) applying ultrasound or electrical energy to the cell; and

(b) contacting the cell, upon conclusion of the application of ultrasound or electrical energy, with a mixture of the nucleic acid molecule and a delivery agent, whereby the nucleic acid molecule is delivered into the cell.

27. (original) The method of claim 26, wherein the agent is a cationic compound.

28. (original) The method of claim 25, wherein the energy is ultrasound.

29. (original) The method of claim 28, wherein prior to applying the ultrasound energy, the cell is contacted with a cavitation compound.

30. (original) The method of claim 1 wherein the cell is a plant cell or an animal cell.

31. (original) The method of claim 1, wherein the cell is selected from the group consisting of a nuclear transfer donor cell, a stem cell, a primary cell, a cell from an immortalized cell line and a cell capable of the generation of a specific organ.

32. (original) The method of claim 1, wherein the cell is selected from the group consisting of a primary cell, an immortalized cell, an embryonic cell, a stem cell, a transformed cell and a tumor cell.

33. (original) The method of claim 1, wherein the cell is selected from the group consisting of a nuclear transfer donor cell, a stem cell, and a cell capable of the generation of a specific organ.

34. (original) A method for delivering a nucleic acid molecule into a cell comprising:

(a) contacting the cell in the absence of the nucleic acid molecule with a delivery agent, and applying ultrasound energy or electrical energy to the cell, wherein the contacting and applying are performed sequentially or simultaneously; and then

(b) contacting the cell with the nucleic acid molecule, whereby the nucleic acid molecule is delivered into the cell.

35. (original) The method of claim 34, wherein the delivery agent comprises a cationic compound.

36. (previously presented) The method of claim 34, wherein the delivery agent is a composition that comprises one or more cationic compounds, wherein the compound is selected from the group consisting of N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), dioleoylphosphatidylethanolamine (DOPE), 2,3-dioleoyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminiumtrifluoroacetate (DOSPA), $C_{52}H_{106}N_6O_4 \cdot 4CF_3CO_2H$, $C_{88}H_{178}N_8O_4S_2 \cdot 4CF_3CO_2H$, $C_{40}H_{84}NO_3P \cdot CF_3CO_2H$, $C_{50}H_{103}N_7O_3 \cdot 4CF_3CO_2H$, $C_{55}H_{116}N_8O_2 \cdot 6CF_3CO_2H$, $C_{49}H_{102}N_6O_3 \cdot 4CF_3CO_2H$, $C_{44}H_{89}N_5O_3 \cdot 2CF_3CO_2H$, $C_{100}H_{206}N_{12}O_4S_2 \cdot 8CF_3CO_2H$, $C_{41}H_{78}NO_8P$, $C_{162}H_{330}N_{22}O_9 \cdot 13CF_3CO_2H$, $C_{43}H_{88}N_4O_2 \cdot 2CF_3CO_2H$, $C_{43}H_{88}N_4O_3 \cdot 2CF_3CO_2H$, and (1-methyl-4-(1-octadec-9-enyl-nonadec-10-enylenyl) pyridinium chloride.

37. (original) The method of claim 34, wherein the delivery agent is 1-methyl-4-(1-octadec-9-enyl-nonadec-10-enylenyl) pyridinium chloride.

38. (original) The method of claim 34, wherein the nucleic acid molecule is greater than about 1 megabase.

39. (previously presented) The method of claim 34, wherein the nucleic acid molecule is selected from the group consisting of an artificial chromosome, a artificial chromosome expression system (ACes) and a natural chromosome or a fragment thereof that is greater than at least about 0.6 megabase.

40. (original) The method of claim 35, wherein the cationic compound is selected from the group consisting of a cationic lipid, a cationic polymer, a mixture of cationic lipids, a mixture of cationic polymers, a mixture of a cationic lipid and a cationic polymer, a mixture of a cationic lipid and a neutral lipid, polycationic lipids, non-liposomal forming lipids, activated dendrimers and a pyridinium chloride surfactant.

41. (original) The method of claim 34, wherein the energy is ultrasound.

42. (previously presented) The method of claim 41, wherein the ultrasound energy is applied to the cell at between about 0.1 and 1 watt/cm², for about 30 seconds to about 5 minutes.

43. (original) The method of claim 41, wherein the ultrasound energy is applied as one continuous pulse or as two or more intermittent pulses.

44. (original) The method of claim 43, wherein:
the pulses are intermittent pulses; and
the intermittent pulses of the ultrasound energy are applied for substantially the same length of time, at substantially the same energy level.

45. (original) The method of claim 43, wherein:
the pulses are intermittent pulses; and
the intermittent pulses vary in energy level, the length of time applied, or energy level and the length of time applied.

46. (original) The method of claim 34, wherein prior to applying the ultrasound energy, the cell is contacted with a cavitation compound.

47. (original) The method of claim 34, wherein the cell is selected from the group consisting of an embryonic stem cell, a nuclear transfer donor cell, a stem cell and a cell capable of the generation of a specific organ.

48. (original) A method for delivering nucleic acid molecule into a cell in a subject comprising:

(a) administering a delivery agent to the subject in the absence of the nucleic acid molecule;

(b) applying ultrasound or electrical energy to the subject after administering the agent; and

(c) administering nucleic acid molecule to the subject upon completion of the application of ultrasound or electrical energy, whereby the nucleic acid molecule is delivered into the cell.

49. (original) The method of claim 48, wherein the agent is a cationic compound.

50. (previously presented) The method of claim 49, wherein administering the cationic compound and the nucleic acid molecule and applying the energy is directly to a localized region of the subject wherein the cell is present.

51. (previously presented) The method of claim 48, wherein the delivery agent is a composition that comprises one or more cationic compounds, wherein the compound is selected from the group consisting of N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), dioleoylphosphatidylethanolamine (DOPE), 2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminiumtrifluoroacetate (DOSPA), $C_{52}H_{106}N_6O_4 \cdot 4CF_3CO_2H$, $C_{88}H_{178}N_8O_4S_2 \cdot 4CF_3CO_2H$, $C_{40}H_{84}NO_3P \cdot CF_3CO_2H$, $C_{50}H_{103}N_7O_3 \cdot 4CF_3CO_2H$, $C_{55}H_{116}N_8O_2 \cdot 6CF_3CO_2H$, $C_{49}H_{102}N_6O_3 \cdot 4CF_3CO_2H$, $C_{44}H_{89}N_5O_3 \cdot 2CF_3CO_2H$, $C_{100}H_{206}N_{12}O_4S_2 \cdot 8CF_3CO_2H$, $C_{41}H_{78}NO_8P$, $C_{162}H_{330}N_{22}O_9 \cdot 13CF_3CO_2H$, $C_{43}H_{88}N_4O_2 \cdot 2CF_3CO_2H$, $C_{43}H_{88}N_4O_3 \cdot 2CF_3CO_2H$, and (1-methyl-4-(1-octadec-9-enyl-nonadec-10-enylenyl) pyridinium chloride.

52. (original) The method of claim 50, wherein the region of the subject is selected from the group consisting of a joint, a tumor, an organ and a tissue.

53. (previously presented) The method of claim 48, wherein the nucleic acid molecule is greater than about 1 megabase.

54. (original) The method of claim 48, wherein the nucleic acid molecule is greater than about 5 megabases.

55. (original) The method of claim 48, wherein the nucleic acid molecule is selected from the group consisting of an artificial chromosome, a satellite artificial chromosome and a natural chromosome or a fragment thereof.

56. (original) The method of claim 49, wherein the cationic compound is selected from the group consisting of a cationic lipid, a cationic polymer, a mixture of cationic lipids, a mixture of cationic polymers, a mixture of a cationic lipid and a cationic polymer, a mixture of a cationic lipid and a neutral lipid, polycationic lipids, non-liposomal forming lipids, activated dendrimers and a pyridinium chloride surfactant.

57. (original) The method of claim 48, wherein the energy is ultrasound and prior to administering the ultrasound energy to the subject, the subject is administered a cavitation compound.

58. Cancelled

59. (previously presented) A method for delivering a large nucleic acid molecule into a cell, comprising:

(a) contacting the nucleic acid molecule with a composition that comprises a cationic lipid, [The method of claim 58,] wherein: the cationic lipid composition comprises 2,3-dioleoyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminiumtrifluoroacetate (DOSPA) and dioleoylphosphatidylethanolamine (DOPE); and the nucleic molecule is at least 5 megabases; and then

(b) contacting the nucleic acid molecule with a cell, wherein steps (a) and (b) are performed simultaneously or sequentially.

61. (previously presented) The method of claim 59, wherein the nucleic acid molecule is a natural chromosome, an artificial chromosome, a fragment of a chromosome, or naked DNA.

62. (previously presented) The method of claim 59, wherein the cell is selected from the group consisting of a plant cell and an animal cell.

63. (previously presented) The method of claim 59, wherein the cell is selected from the group consisting of a primary cell, an immortalized cell, an embryonic cell, a stem cell, a transformed cell and a tumor cell.

64. (previously presented) The method of claim 59, wherein the nucleic acid molecule is contacted with the cell in vitro, ex vivo or in vivo.

65. (previously presented) A method for delivering a nucleic acid molecule into a cell in a subject comprising:

(a) mixing the nucleic acid molecule with a delivery agent;

(b) administering the mixture of nucleic acid molecule and agent to the subject; and

(c) applying ultrasound or electrical energy to the subject, whereby the nucleic acid molecule is delivered into the cell to a greater extent than using the agent or energy alone.

66. (original) The method of claim 65, wherein the agent is a cationic compound.

67. (previously presented) The method of claim 66, wherein the cationic compound and the nucleic acid molecule mixture are applied locally.

68. (original) The method of claim 67, wherein the mixture is applied to a joint, a tumor, an organ or a tissue.

69. (original) The method of claim 65, wherein the nucleic acid molecule is greater than about 1 megabase.

70. (original) The method of claim 66, wherein the cationic compound is selected from the group consisting of a cationic lipid, a cationic polymer, a mixture of cationic lipids, a mixture of cationic polymers, a mixture of a cationic lipid and a cationic polymer, a mixture of a cationic lipid and a neutral lipid, polycationic lipids, non-liposomal forming lipids, activated dendrimers, and a pyridinium chloride surfactant.

71. (previously presented) The method of claim 65, wherein the nucleic acid molecule is selected from the group consisting of an artificial chromosome, an artificial chromosome expression system (ACes), a natural chromosome or a fragment thereof that is greater than at least about 0.6 megabase.

72. (original) The method of claim 65, wherein the nucleic acid molecule is a natural chromosome, an artificial chromosome, a fragment of a chromosome or naked DNA that is greater than at least about 0.6 megabase in size.

73. (original) A method for delivering nucleic acid molecule into a cell in a subject comprising:

- (a) applying ultrasound or electrical energy to subject; and
- (b) administering to the subject a nucleic acid molecule and a delivery agent, upon conclusion of the application of ultrasound or electrical energy, whereby the nucleic acid molecule is delivered into the cell, wherein the delivery agent and nucleic acid are administered sequentially or as a single composition.

74. (original) The method of claim 73, wherein the delivery agent is administered, upon conclusion of the application of ultrasound or electrical energy followed by administration of the nucleic acid molecule, whereby the nucleic acid molecule is delivered into a cell.

75. (original) The method of claim 73, wherein prior to applying the ultrasound energy, the subject is administered a cavitation compound.

76. (original) The method of claim 75, wherein the energy is ultrasound and prior to applying the ultrasound energy, the subject is administered a cavitation compound.

77. (original) The method of claim 63, wherein the agent is a cationic compound.

78. (original) The method of claim 77, wherein the cationic compound is selected from the group consisting of a cationic lipid, a cationic polymer, a mixture of cationic lipids, a mixture of cationic polymers, a mixture of a cationic lipid and a cationic polymer, a mixture of a cationic lipid and a neutral lipid, polycationic lipids, non-liposomal forming lipids, activated dendrimers, and a pyridinium chloride surfactant.

79. (previously presented) The method of claim 73, wherein the delivery agent is a composition that comprises one or more cationic compounds, wherein the compound is selected from the group consisting of N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), dioleoylphosphatidylethanolamine (DOPE), 2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminiumtrifluoroacetate (DOSPA), $C_{52}H_{106}N_6O_4 \cdot 4CF_3CO_2H$, $C_{88}H_{178}N_8O_4S_2 \cdot 4CF_3CO_2H$, $C_{40}H_{84}NO_3P \cdot CF_3CO_2H$, $C_{50}H_{103}N_7O_3 \cdot 4CF_3CO_2H$, $C_{55}H_{116}N_8O_2 \cdot 6CF_3CO_2H$, $C_{49}H_{102}N_6O_3 \cdot 4CF_3CO_2H$, $C_{44}H_{89}N_5O_3 \cdot 2CF_3CO_2H$, $C_{100}H_{206}N_{12}O_4S_2 \cdot 8CF_3CO_2H$, $C_{41}H_{78}NO_8P$, $C_{162}H_{330}N_{22}O_9 \cdot 13CF_3CO_2H$, $C_{43}H_{88}N_4O_2 \cdot 2CF_3CO_2H$, $C_{43}H_{88}N_4O_3 \cdot 2CF_3CO_2H$, and (1-methyl-4-(1-octadec-9-enyl-nonadec-10-enylenyl) pyridinium chloride).

80. (original) A method for delivering nucleic acid molecule into a cell in a subject comprising:

- (a) applying ultrasound or electrical energy to the subject; and
- (b) administering to the subject the nucleic acid molecule upon conclusion of the application of ultrasound or electrical energy, whereby the nucleic acid molecule is delivered into the cell.

81. (original) The method of claim 80, wherein the energy is ultrasound and prior to applying the ultrasound energy, the subject is administered a cavitation compound.

82. (original) The method of claim 80, wherein the agent comprises a cationic compound.

83. (original) The method of claim 80, wherein the nucleic acid molecule is a natural chromosome, an artificial chromosome, a fragment of a chromosome or naked DNA that is greater than at least about 0.6 megabase in size.

84.-140. Cancelled

141. (previously presented) A kit for delivering nucleic acids into cells, comprising:

- a composition comprising an artificial chromosome;
- a delivery agent that comprises a composition comprising a delivery agent;
- reagents for performing sonoporation or electroporation; and
- optionally instructions for delivering nucleic acids into cells.

142. (original) The kit of claim 141, wherein the delivery agent comprises a compound is selected from the group consisting of a cationic lipid, a cationic polymer, a mixture of cationic lipids, a mixture of cationic polymers, a mixture of a cationic lipid and a cationic polymer, a mixture of a cationic lipid and a neutral lipid, polycationic lipids, non-liposomal forming lipids, activated dendrimers, and a pyridinium chloride surfactant.

143. (previously presented) The kit of claim 142, wherein the compound is selected from the group consisting of N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), dioleoylphosphatidylethanolamine (DOPE), 2,3-dioleoyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminiumtrifluoroacetate (DOSPA), $C_{52}H_{106}N_6O_4 \cdot 4CF_3CO_2H$, $C_{88}H_{178}N_8O_4S_2 \cdot 4CF_3CO_2H$, $C_{40}H_{84}NO_3P \cdot CF_3CO_2H$, $C_{50}H_{103}N_7O_3 \cdot 4CF_3CO_2H$, $C_{55}H_{116}N_8O_2 \cdot 6CF_3CO_2H$, $C_{49}H_{102}N_6O_3 \cdot 4CF_3CO_2H$, $C_{44}H_{89}N_5O_3 \cdot 2CF_3CO_2H$, $C_{100}H_{206}N_{12}O_4S_2 \cdot 8CF_3CO_2H$, $C_{41}H_{78}NO_8P$, $C_{162}H_{330}N_{22}O_9 \cdot 13CF_3CO_2H$, $C_{43}H_{88}N_4O_2 \cdot 2CF_3CO_2H$, $C_{43}H_{88}N_4O_3 \cdot 2CF_3CO_2H$, and (1-methyl-4-(1-octadec-9-enyl-nonadec-10-enylenyl) pyridinium chloride.

144. (previously presented) The method of claim 1, wherein the nucleic acid molecule is about 10 megabases to about 450 megabases.

145. (previously presented) The method of claim 1, wherein the nucleic acid molecule is about 90 megabases to about 120 megabases.

146. (previously presented) The method of claim 1, wherein the nucleic acid molecule is about 15 megabases to about 50 megabases.

147. (previously presented) The method of claim 59, wherein the nucleic acid molecule is about 10 megabases to about 450 megabases.

U.S. Application Serial No. 09/815,981 Pending Claims:

1. (Currently Amended) A method for detecting or determining delivery and expression of a nucleic acid introduced into a cell comprising:
introducing intact and condensed labelled large nucleic acid molecules that encode a reporter gene into cells, wherein the nucleic acid molecules remain intact and condensed after delivery;
detecting labelled cells as an indication of delivery of the nucleic acid into a cell; and
measuring the product of the reporter gene as an indication of DNA expression in the cell, whereby delivery and expression of nucleic acid molecules in the cell is detected or determined.
2. (original) The method of claim 1, wherein the labelled cells are detected by flow cytometry, fluorimetry, cell imaging or fluorescence spectroscopy.
3. (Previously presented) The method of claim 1, wherein the labelled cells are detected by flow cytometry.
4. (original) The method of claim 1, wherein the nucleic acid molecule is DNA.
5. (original) The method of claim 1, wherein the label is iododeoxyuridine (IdU or IdUrd) or bromodeoxyuridine (BrdU).
6. (original) The method of claim 1, wherein the reporter gene encodes a fluorescent protein, or enzyme or antibody.
7. (original) The method of claim 6, wherein the enzyme is a luciferase, β -galactosidase or alkaline phosphatase.
8. (original) The method of claim 6, wherein the fluorescent protein is a red, green or blue fluorescent protein.
9. (previously presented) The method of claim 6, wherein the step of introducing labelled large nucleic acid molecules comprises contacting the nucleic acid molecules with a delivery agent that comprises a cationic compound.
10. (previously presented) The method of claim 9, wherein the compound is selected from the group consisting of N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), dioleoylphosphatidylethanolamine (DOPE), 2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminiumtrifluoroacetate (DOSPA), $C_{52}H_{106}N_6O_4 \cdot 4CF_3CO_2H$, $C_{88}H_{178}N_8O_4S_2 \cdot 4CF_3CO_2H$, $C_{40}H_{84}NO_3P \cdot CF_3CO_2H$, $C_{50}H_{103}N_7O_3 \cdot 4CF_3CO_2H$, $C_{55}H_{116}N_8O_2 \cdot 6CF_3CO_2H$, $C_{49}H_{102}N_6O_3 \cdot 4CF_3CO_2H$, $C_{44}H_{89}N_5O_3 \cdot 2CF_3CO_2H$, $C_{41}H_{78}NO_8P$, $C_{100}H_{206}N_{12}O_4S_2 \cdot 8CF_3CO_2H$, $C_{162}H_{330}N_{22}O_9 \cdot 13CF_3CO_2H$, $C_{43}H_{88}N_4O_2 \cdot 2CF_3CO_2H$, $C_{43}H_{88}N_4O_3 \cdot 2CF_3CO_2H$ and (1-methyl-4-(1-octadec-9-enyl-nonadec-10-enylenyl) pyridinium chloride.
11. (previously presented) The method of claim 1, wherein the nucleic acid molecules are natural chromosomes, artificial chromosomes, fragments of a chromosome or naked DNA that is greater than at least about 0.6 megabase in size.
12. (original) The method of claim 1, wherein the nucleic acid molecules are artificial chromosomes, plasmids, chromosome fragments, naked DNA, or natural chromosomes.

13. (previously presented) The method of claim 1, wherein the nucleic acid molecules are artificial chromosome expression systems (ACes).

14. (original) The method of claim 1, wherein the cells are eukaryotic cells.

15. (previously presented) The method of claim 14, wherein the cells are primary cells, cell lines, plant cells, or animal cells.

16. (previously presented) The method of claim 14, wherein the cells are stem cells, nuclear transfer donor cells, tumor cells or transformed cells.

Claims 17-29 Cancelled

30. (previously presented) The method of claim 1, wherein the cell is selected from the group consisting of a primary cell, an immortalized cell, an embryonic cell, a stem cell, a transformed cell and a tumor cell.

Claims 31-32 Cancelled

33. (new) The method of claim 1, wherein the labelled large nucleic acid is labelled by metabolic labelling or in vivo labelling.